OBSERVATIONS ON THE DEVELOPMENT OF HOOKWORM LARVAE

PART II

BY

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The technique employed in the following experiments is exactly the same as that described in Part I of this paper, and the results of the experiments are all expressed as the number of larvae isolated per gram of faeces employed. It will also be noted that in the various series of experiments the numbers of larvae recovered are very different ; this is because faeces from different individuals were used.

EXPERIMENT 4.

The effect of urine on mixtures of soil and faeces.

In this experiment three grams of faeces were mixed with laterite in the ordinary way, being kept moist by the daily addition, for a period of seven days, of sufficient fresh urine for the purpose. Control cultures had equal quantities of water added.

SERIES 1.

Urine-moistened cultures produced	6 larvae.
Control cultures produced	1,736 larvae.

SERIES 2.

Urine-moistened cultures produced	22 larvae.
Control cultures produced	144 larvae.

The larvae isolated from the urine-moistened cultures were very small and were degenerate in appearance, and it is doubtful if any of them were infective. They were placed in tap water to test their viability, and all were dead within forty-eight hours, whereas larvae from the controls showed no diminution in numbers living or in activity, at the end of the same period.

EXPERIMENT 5.

The effect of the immersion of faeces in different liquids for varying periods.

In this series of experiments the faeces were immersed in the different liquids for the times stated ; they were then mixed with laterite and cultured for seven days in the routine manner.

EXPERIMENT 5a.

Effect of prolonged immersion in tap water without disintegration of faeces.

Portions of faeces, each of about ten grams in weight, were tied in small pieces of muslin and each packet was placed in a separate beaker of tap water. The packets of faeces floated partly submerged in the water. At the end of the periods stated in the table below, the water in which the faeces were immersed was examined for the presence of larvae, three grams of faeces were taken and mixed with laterite for culture, and the balance of the faeces was placed direct into the isolation apparatus to see if larvae were present.

No.	Time immersion was continued	Larvae in water of immersion	Eggs in faeces	Larvae in faeces	Yield of larvae from culture
1	1 week	0	+	0	37
2	2 weeks	0	+	0	44
3	3 weeks	0	+	0	53
4	4 weeks	0	+	0	35
5	5 weeks	0	+	0	39

Even after five weeks' immersion the faeces looked just as fresh as when first placed in the muslin and the eggs showed no apparent development.

EXPERIMENT 5b.

The effect of the immersion of faeces in different liquids for varying periods.

Equal portions of faeces from a well-mixed stool were placed in beakers ; they were not tied in muslin, so the various liquids added could act freely on them. The liquids used were tap water, a disinfectant containing 1 per cent. free chlorine, sea water, and fresh urine. The faeces became saturated and sank to the bottom of the beakers in about twenty-four hours. After varying periods, indicated in the table below, the liquids were drawn off with a pipette as completely as possible. None of the liquids contained any larvae. Portions of the faeces, three grams in weight, were now mixed with laterite and were cultured for seven days. During the period of growth they were all kept moist with tap water, irrespective of the liquid in which they were originally immersed ; by keeping the cultures under identical conditions in this manner, the lethal effect of the liquids on the eggs and not on the young larvae could be determined.

Liquid in which immersed	Number of days immersion lasted				
	1	2	4	7	9
Tap water	4,337	Culture lost	3,350	2,460	2,837
Sea water	4,730	Culture lost	4,223	1,393	1,287
Disinfectant	5,640	2,967	2,460	2,260	2,857
Urine	4,680	4,607	3,983	2,837	2,360
	Larvae isolated per gram of faeces				

Control cultures put up on the day on which immersion was commenced produced 6,097 larvae. The greater number of larvae in the controls is probably due not to the death of some eggs after only one day's immersion, but rather to the unavoidable dilution of the faeces after immersion.

Experiment 5c.

Effect of immersion for prolonged periods in various liquids.

Except that the immersion of the faeces in the liquids was continued for longer periods this experiment is identical with Experiment 5b. Moulds growing on the surfaces of the liquids were removed with a section-lifter, care being taken not to disturb the faeces lying at the bottom of the beakers.

Fluid in which immersed	Number of days immersion lasted					
	7	14	18	21	28	35
Tap water	177	127	150	138	27	0
Sea water	127	100	80	27	0	0
Urine	140	0	0	0	0	0
	Larvae isolated per gram of faeces					

Cultures made from the same specimen of faeces on the day on which immersion was commenced produced 583 larvae. As in Experiment 5b, the greater number of larvae recovered from the controls is to some extent probably due to dilution of the faeces, but the difference between the controls and immersed faeces is more marked in this instance because the first examination was not made until after a week's immersion, at which time, judging by analogy with Experiment 5b, about half the eggs would be dead even in tap water.

EXPERIMENT 5d.

Effect of mixing faeces with soil before immersion.

Portions of faeces, three grams in weight, were mixed with 25 c.c. of laterite or sea sand, placed in beakers, and covered with either tap water, sea water or urine. The beakers were allowed to stand for fourteen days; this time was chosen on account of the results obtained in Experiment 5c. At the end of this time the liquids were pipetted off and examined for the presence of larvae, but none were found in any instance. The whole of the soil and faeces mixtures was now transferred to wire baskets and they were at once tested by hot water isolation. The baskets were then allowed to drain off any excess of water and were kept for a further seven days, being kept moist with tap water meanwhile. At the end of this time they were again subjected to isolation with hot water. The results are given in the table below.

	Liquid in which immersed		
	Tap water	Sea water	Urine
First isolation— Laterite mixture	0	0	0
Sea sand mixture	0	0	0
Second isolation— Laterite mixture	80	253	0
Sea sand mixture	127	201	0
	Larvae isolated per gram of faeces		

Control cultures made on the day on which the experiment was commenced produced 820 larvae per gram of faeces. In this instance dilution of the faeces does not partially explain the difference, for the whole of the contents of each beaker was subjected to isolation, and it was noted that no eggs were lost from the culture during the first isolation.

EXPERIMENT 5e.

Effect of saturation in different liquids without immersion in them.

This experiment is the same as Experiment 3b in Part I of this paper, except that urine was used in addition to tap water and sea water. For the sake of convenient comparison the figures in Experiment 3b are here reproduced, together with those obtained from the use of urine under the same conditions.

Series 1 (kept for 7 days).

	Liquid in which saturated		
	Tap water	Sea water	Urine
First isolation— Laterite	50	53	53
Sea sand	7	17	0
Second isolation— Laterite	67	7	7
Sea sand	197	153	0

Series 2 (kept for 14 days).

	Liquid in which saturated		
	Tap water	Sea water	Urine
First isolation—			
Laterite	230	137	0
Sea sand	20	7	0
Second isolation—			
Laterite	0	0	0
Sea sand	203	17	0
	Larvae isolated per gram of faeces		

Control cultures of the same faeces put up on the day on which the experiment was commenced produced 820 larvae per gram of faeces.

DISCUSSION

This series of experiments shows that urine has a definite effect in destroying both hookworm eggs and freshly-hatched larvae. In Experiments 5b and 5c it will be noted that it is somewhere between nine and fourteen days before all the hookworm eggs in faeces are killed by urine. In Experiment 4 the eggs must have been in a condition to hatch out, so it must be assumed that urine-moistened soil will kill young larvae. Minagawa (1919), working with *Ancylostomum caninum,* showed indirectly that urine killed the larvae of this species, for he failed to infect young dogs that were placed on soil that had been heavily infected with faeces containing eggs of *A. caninum,* and which had been moistened with urine previously, whereas in his control experiments the dogs became infected. The inference drawn by Minagawa was that the larvae of *A. caninum* were killed by contact with human urine, and the writer's experiment shows that the same applies to the larvae of hookworms infecting human beings.

In Experiment 5a, it will be seen that the packets of faeces were only partly submerged in water, and the result was that after five weeks there was no death of hookworm eggs ; in Experiment 5c, however, in which the faeces were totally excluded from contact with air, all the eggs failed to hatch after the same period of immersion.

Khalil (1922) found apparently normal undeveloped hookworm eggs in the effluent of septic tanks in British Guiana, and he concludes from this that septic tanks of the type investigated are of no use in preventing the spread of Ankylostomiasis except by concentrating the infective material into one channel. As there was apparently no way of knowing how many eggs entered the tanks, it is not possible to say if any were killed during their passage through the system. Experiment 5b in the present series shows that after four days' complete immersion in water about 25 per cent. of the eggs were destroyed, and Experiment 5c shows that there is a steady diminution in the number of viable eggs recoverable from water as immersion is continued, until after a period of five weeks all the eggs seem to be dead. From these observations it seems safe to conclude that septic tanks would have some effect in reducing hookworm infection, the actual reduction being directly dependent on the time the faeces remained in the tank.

REFERENCES

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